

# Gall Structure Affects Ecological Associations of *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae)

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**ABSTRACT** Gall wasps (Hymenoptera: Cynipidae) induce structures (galls) on their host plants that house developing wasps and provide them with protection from natural enemies. The Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, is an invasive pest that is destructive to chestnut (*Castanea* spp.). An improved understanding of the interactions among *D. kuriphilus*, its host, and its natural enemies is critical for the development of effective management strategies against this pest. The objective of our study was to evaluate the *D. kuriphilus* community interactions, and relate these interactions to variations among gall traits. Galls were collected from four locations throughout the eastern United States from May (gall initiation) through August (after gall wasp emergence), and January. Gall characteristics (volume, weight, and schlerenchyma layer thickness), gall inhabitants (*D. kuriphilus*, parasitoids, and chamber fungi), and other community associates (insect herbivores and lesions thought to be caused by endophytes) were evaluated and correlated using canonical correlation analyses. The primary mortality factors for *D. kuriphilus* were parasitism, gall chamber-invading fungi, and failure of adult gall wasps to emerge. Larger gall size and thicker schlerenchyma layers surrounding the larval chambers were negatively correlated with parasitoids and chamber fungi, indicating these gall traits are important defenses. External fungal lesions and insect herbivory were positively correlated with the absence of *D. kuriphilus* within galls. This study provides support for the protective role of cynipid galls for the gall inducer, identifies specific gall traits that influence gall wasp mortality, and improves our knowledge of *D. kuriphilus* ecology in North America.

**KEY WORDS** American chestnut, Chinese chestnut, chestnut weevil, *Torymus sinensis*, *Ormyrus labotus*

Gall wasps (Hymenoptera: Cynipidae) induce abnormal growths (galls) on their host plants in which the immature gall wasp stages develop (Kinsey 1920, Felt 1940). Galls are active nutritive sinks that provide developing wasps with a continuous nutritive supply (Hartley and Lawton 1992, Hartley 1998, Allison and Schultz 2005, Cooper and Rieske 2009). The formation of galls is postulated to be consistent with the enemy hypothesis, which states that galls provide the gall inducer with protection from predators, parasitoids, pathogens, and incidental grazing by insect herbivores (Cornell 1983, Price et al. 1987, Taper and Case 1987, Hartley and Lawton 1992, Schultz 1992). Nonetheless, gall wasps are highly susceptible to a broad range of gall-attacking parasitoids (Shorthouse 1973, 1993; Ito and Hijii 2002; Cooper and Rieske 2007) and are also subject to pathogen attack (Wilson 1995, Cooper and Rieske 2007).

Most cynipids are not considered economic pests (Kinsey 1935, Stone et al. 2002). A notable exception

is *Dryocosmus kuriphilus* Yasumatsu, which induces galls on the actively growing shoots of all chestnut species (*Castanea* spp., Fagales: Fagaceae). Galling by *D. kuriphilus* prevents or inhibits shoot development and flowering and can stress chestnut trees enough to contribute to mortality (Kato and Hijii 1997, Aebi et al. 2006). Chestnut is an important global nut commodity and ornamental nursery tree. Additionally, American chestnut (*Castanea dentata* Marshall) was once a codominant canopy tree species in eastern North America until the accidental introduction of the chestnut blight fungus, *Cryphonectria* (*Endothia*) *parasitica* (Murr) Barr (Diaporthales: Valsaceae). The blight fungus forms stem cankers on susceptible chestnut and kills plant tissues distal to infection (Paillet 1982, 2002). Efforts are underway to breed blight resistance from Chinese chestnut, *Cryphonectria mollissima* Blume, into American chestnut for restoration purposes (Hebard 1994). Damage caused by *D. kuriphilus* galling poses a significant threat to cultivated chestnuts and American chestnut restoration (Kato and Hijii 1997, Anagnostakis 2001). An improved understanding of *D. kuriphilus* ecology in North America is critical to the development of effective management strategies for commercial chestnut and for efforts to restore American chestnut to Appalachian forests.

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A native of Asia, *D. kuriphilus* was accidentally introduced to North America in 1974 (Payne et al. 1975), and most recently to Europe (Aebi et al. 2006). Parthenogenetic adults emerge in midsummer and oviposit on new buds. Eggs hatch soon after oviposition, and larvae remain inconspicuously within the bud throughout the winter. Larvae induce the formation of spherical galls on developing leaves and shoots in conjunction with spring budbreak, and then complete development by midsummer. In North America, *D. kuriphilus* is parasitized primarily by the introduced Asian parasitoid, *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) (Cooper and Rieske 2007). In some regions of North America, the native parasitoid of cynipid wasps, *Ormyrus labotus* Walker (Hymenoptera: Ormyridae), also inhabits *D. kuriphilus* galls (Cooper and Rieske 2007). Gall chambers are sometimes filled with an unidentified fungus, which is indicative of wasp mortality (Cooper and Rieske 2007, 2009). Damage to gall exteriors is caused by the formation of lesions thought to be induced by endophytes, by feeding from the lesser chestnut weevil (*Curculio sayi* Gyllenhal, Coleoptera: Curculionidae) (Cooper and Rieske 2007), and to a lesser extent by incidental grazing from generalist insect herbivores (Schultz 1992; W.R.C., unpublished data).

Gall location and locularity are influenced by maternal gall wasps, whereas gall initiation and maintenance are influenced by larvae (Bailey et al. 2009). However, galls are formed entirely of plant tissue; thus, gall initiation and growth are also influenced by host plant traits and/or environmental factors that affect plant phenotypes (Washburn and Cornell 1981, Eliason and Potter 2000, Sumerford et al. 2000, Ito and Ozaki 2005, Anagnostakis et al. 2009). Intraspecific variations among galls, whether controlled by the gall wasp or influenced by plant phenotypes, may affect interactions between cynipids and their community associates, and/or influence the protective roles of galls (Taper and Case 1987, Ito and Hijii 2002, Ito and Ozaki 2005; unpublished data).

The overall goal of our study was to investigate ecological interactions among *D. kuriphilus*, chestnut, and the North American community associated with *D. kuriphilus*, and to relate these ecological interactions to variations in gall characteristics. Specific objectives were to evaluate the following: 1) the gall community succession in different regions of North America; 2) the relationships between gall characteristics and gall inhabitants or external associates; and 3) the effects of external associates on gall inhabitants.

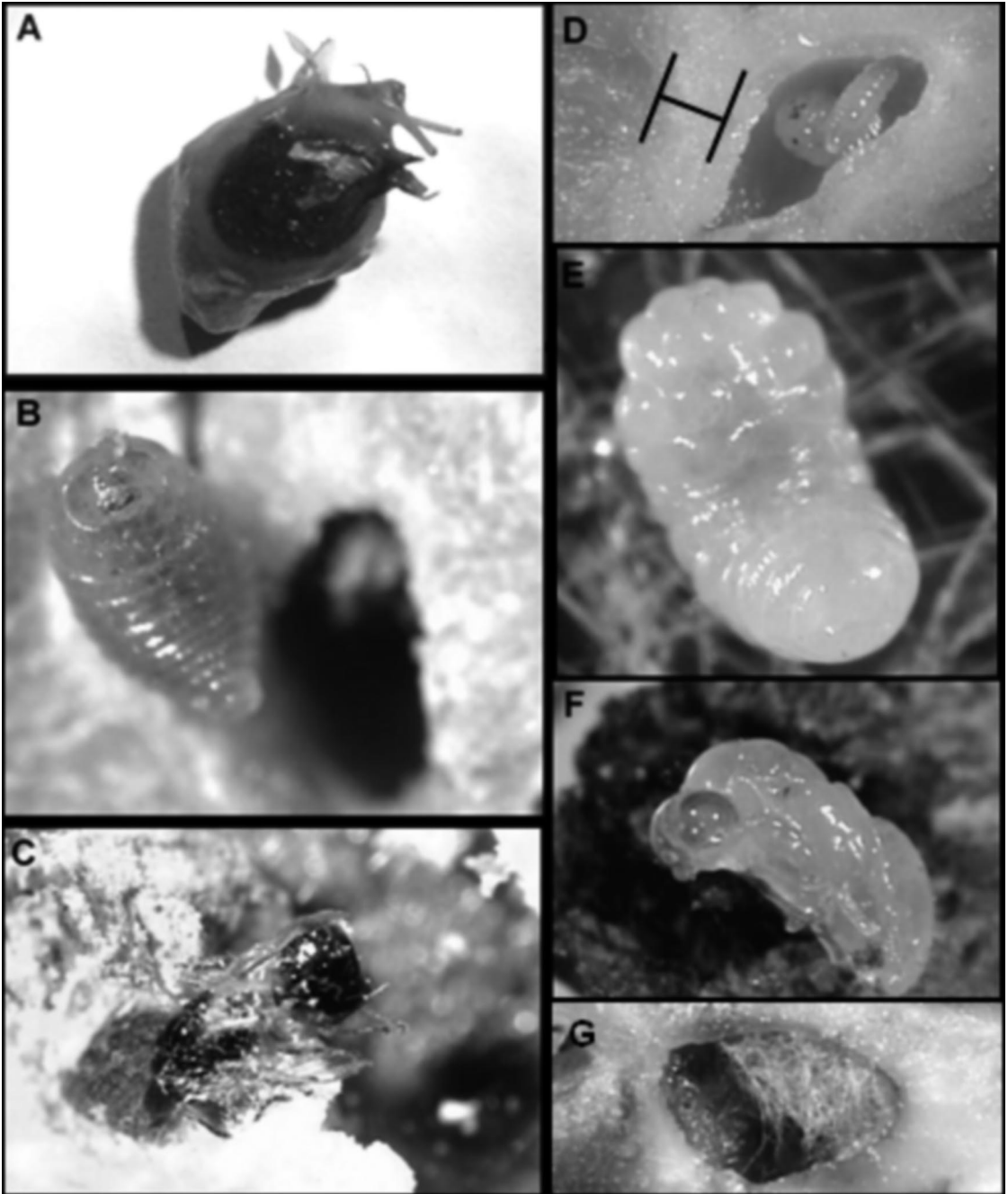
### Materials and Methods

**Study Locations.** Four geographically distinct locations were selected for gall collections, including two orchard settings (experimental orchard in Meadowview, VA, and a commercial orchard in Hiram, OH), ornamental Chinese chestnuts in a suburban setting (Broadview Heights, OH), and naturally occurring American chestnuts persisting in a mosaic forest despite blight infection (Bowling Green, KY). The

Meadowview, Broadview Heights, and Bowling Green collection sites were described previously (Cooper and Rieske 2007). The Hiram collection site is located in Portage County, Ohio (41° 19' N latitude, 81° 11' W longitude) ≈45 km east of Broadview Heights, and consists of even-aged hybrid chestnuts planted for commercial chestnut production. The gall wasp infestation at Hiram was first noticed in 2005 (Stehli 2006).

**Gall Collections and Assessments.** Galls were collected from each location in the third week of each month from May through August 2006, with a single winter collection in January 2007. In Bowling Green, ≈10–15 galls per tree were collected from two naturally occurring American chestnuts on each collection date ( $n = 21$  in May and June,  $n = 27$  in July,  $n = 16$  in August, and  $n = 30$  in January). In Broadview Heights, ≈10–15 galls per tree were collected from three Chinese chestnuts on each collection date ( $n = 43$  in May,  $n = 30$  in June,  $n = 31$  in July,  $n = 27$  in August, and  $n = 30$  in January). In Hiram, ≈5 galls per tree were collected from 10 advanced hybrid chestnuts (various combinations of American, Chinese, European, and Japanese chestnuts) on each collection date ( $n = 55$  in May,  $n = 47$  in June,  $n = 62$  in July,  $n = 44$  in August, and  $n = 50$  in January). In Meadowview, ≈5–10 galls per tree were collected from five American × Chinese hybrid chestnuts on each collection date ( $n = 37$  in May,  $n = 51$  in June,  $n = 36$  in July,  $n = 51$  in August, and  $n = 50$  in January). Galls were excised from arbitrary locations within each tree, placed in plastic bags, kept on ice during transport, and stored in  $-20^{\circ}\text{C}$ .

Each gall was identified as either a stem gall (occurring on the chestnut shoot with ≈2–6 attached leaves) or a leaf gall (occurring along the leaf midvein). The volume ( $\text{mm}^3$ ) of each gall was estimated using the equation for an ellipse volume ( $[\text{four-thirds}] \times \pi \times \text{radius1} \times \text{radius2} \times \text{radius3}$ ), where radii 1–3 were measured with calipers on three opposite planes across the gall (length and two right-angle measurements for width) (Cooper and Rieske 2009). Gall exteriors were visually assessed for weevil herbivory (number of feeding sites per gall), presence of black fungal lesions (Fig. 1A), and presence of damage caused by grazing insect herbivores. Each gall was then dissected under a stereomicroscope with a ×160 magnification. The number of chambers per gall was counted, and the contents of each were identified as either larva (Fig. 1B), pupa, or adult *D. kuriphilus* (Fig. 1C); *D. kuriphilus* and parasitoid larva occurring simultaneously within the chamber (Fig. 1D); larva (Fig. 1E), pupa (Fig. 1F), or adult parasitoid occurring within the chamber without *D. kuriphilus*; chamber fungi (Fig. 1G); empty chamber without an emergence hole; and empty chamber with an emergence hole. Gall chambers were cross-sectioned, and the thickness of the sclerenchyma layers surrounding the larval chambers (visible in Fig. 1D) was measured using an ocular micrometer (Cooper and Rieske 2009). The proportion of chambers containing gall wasps, parasitoids, or internal fungi, or that were empty was calculated for each gall by dividing the



**Fig. 1.** North American community associates of *D. kuriphilus*. (A) Black lesions on the exterior of *D. kuriphilus* chestnut galls that are thought to be produced by endophytes. (B) *D. kuriphilus* larva and (C) unemerged *D. kuriphilus* adult. (D) Parasitoid larvae occupying a gall chamber with *D. kuriphilus*, and (E) parasitoid larvae and (F) parasitoid pupae individually occupying a gall chamber. (E) The boundaries of the schlerenchyma layer marked. (G) Empty chamber filled with white mycelia.

respective count by the number of gall chambers. In addition to chamber inhabitants, the number of acarid mites (Acari: Acaridae) inhabiting the parenchyma tissues and chambers of galls was counted.

**Statistical Analyses.** Individual galls were considered experimental units. All statistical analyses were

performed using SAS 9.01 (SAS Institute, Cary, NC). Values for gall volume, schlerenchyma thickness, weevil herbivory, and number of mites per gall were natural log transformed to stabilize variances. Proportional data (chamber contents) were arcsine-square root transformed. Untransformed data are presented

Table 1. Analyses of morphological characteristics of *D. kuriphilus* galls collected from four locations in the eastern United States

| Collection site       | Chestnut             | N   | No. chambers/gall              | Gall vol (mm <sup>3</sup> )     | Ratio of stem galls to leaf galls  |
|-----------------------|----------------------|-----|--------------------------------|---------------------------------|------------------------------------|
| Broadview Heights, OH | Chinese              | 161 | 5.4 ± 0.3a                     | 1756.9 ± 134.1a                 | 77.0 ± 3.3ab                       |
| Hiram, OH             | Hybrids <sup>a</sup> | 258 | 2.6 ± 0.1c                     | 472.9 ± 23.2b                   | 81.4 ± 2.4a                        |
| Meadowview, VA        | Hybrids <sup>b</sup> | 215 | 2.8 ± 0.1c                     | 318.8 ± 16.8b                   | 70.7 ± 3.1b                        |
| Bowling Green, KY     | American             | 115 | 3.5 ± 0.2b                     | 373.8 ± 27.4b                   | 79.1 ± 3.8ab                       |
| ANOVA                 |                      | 749 | $F_{3, 742} = 52.72; P < 0.01$ | $F_{3, 742} = 114.23; P < 0.01$ | $\chi^2_{3, 742} = 7.92; P < 0.04$ |

Values within columns with different letters are significantly different at  $\alpha = 0.05$ .  
<sup>a</sup> Advanced hybrids consisting of various chestnut lineages, including American, European, Chinese, and Japanese chestnuts.  
<sup>b</sup> Chinese × American chestnut hybrids.

in tables and graphs. For all analyses, values were considered significantly different at  $\alpha = 0.05$ .

The number of chambers per gall does not change after gall induction. Therefore, these values were pooled over the course of the experiment and analyzed with collection site as the dependent variable, followed by Tukey's honestly significant difference adjusted post hoc mean separations. Gall volume, schlerenchyma layer thickness, and weevil herbivory (feeding sites per gall) were analyzed using a factorial analysis of variance (ANOVA) with collection date and site as main effects. Gall chamber inhabitants (gall wasps, parasitoids, empty chambers, emergence holes, internal fungi, mites) were analyzed using a factorial multivariate ANOVA with collection date and site as main effects and the number of chambers per gall as a covariate. Roy's greatest root was used to test the significance of the multivariate model. This test provides the greatest power compared with other multivariate ANOVA measures when different *F*-statistics are calculated among the different measures (Scheiner 2001). Multivariate analyses were followed with univariate tests for each variable when significant multivariate effects were detected. Tukey's honestly significant difference adjusted pairwise comparisons were used to compare treatment means within each month when significant date or site × date interactions were detected.

For the categorical data (incidence of stem galls, gall lesions, and herbivore grazing), the effects of collection site, date, and their interaction were analyzed using binomial logistic regression (PROC LOGISTIC). When significant date effects and site × date interactions were not detected, monthly data were pooled and logistic regression was used to analyze variables between collection sites. Data were analyzed within collection date when significant date effects or site × date interactions were detected.

Canonical correlation analyses (PROC CANCORR) extract the maximum relationship between two data sets into new variables. Each new relationship results in canonical variables that are orthogonal to the original variables. Canonical correlation analyses within each month were used to evaluate correlations as follows: 1) among gall characteristics (gall volume and chamber schlerenchyma thickness) and occurrence of internal gall associates (*D. kuriphilus*, parasitoids, emerged wasps, empty chambers, and chamber fungi); 2) among gall characteristics (gall

volume, schlerenchyma thickness, stem versus leaf galls) and occurrence of external gall associates (external lesions, weevil herbivory, herbivore grazing); and 3) among external associates and internal associates. The different life stages (larvae, pupae, and adults) for both *D. kuriphilus* and parasitoids were pooled for canonical correlations.

Results

**Gall Characteristics.** Galls collected from different collection sites varied with respect to the number of chambers, gall volume, and the ratio of stem to leaf galls (Table 1). There was no date effect for gall volume ( $F = 0.67$ ;  $df = 4, 741$ ;  $P = 0.61$ ), so values across dates were pooled and compared among collection sites. Galls collected in Broadview Heights (cultivated Chinese chestnut) were significantly larger and contained more chambers compared with galls collected from other locations (Table 1). Galls collected in Bowling Green (naturally occurring American chestnut) contained significantly more chambers compared with galls collected from Meadowview and Hiram (hybrid chestnuts), but gall volume did not differ between these two sites (Table 1). There was a significantly greater proportion of stem galls on trees in Hiram compared with trees in Meadowview, but the stem gall versus leaf gall ratio did not differ on trees in Hiram, Bowling Green, and Broadview Heights (Table 1).

Analysis of schlerenchyma layer thickness revealed a significant site × date interaction ( $F = 10.8$ ;  $df = 12, 729$ ;  $P < 0.01$ ) (data not shown). The schlerenchyma layer thickness declined throughout the collection period (May–January) at all locations, but became difficult to identify as galls aged. The schlerenchyma layer was thickest in galls collected from Broadview Heights compared with other locations in May through August, and thinnest in galls collected from Bowling Green compared with other locations in May through July.

**Gall Inhabitants and Associates.** Multivariate ANOVA of gall inhabitants revealed significant differences in the patterns of gall parameters among collection dates and sites ( $F = 91.88$ ;  $df = 12, 719$ ;  $P < 0.01$ ). Univariate analyses indicated each gall dissection variable differed among collection dates and sites, and with the exception of adult parasitoid incidence, demonstrated significant date × site interactions (Ta-



Table 2. Univariate full factorial analyses of internal gall associates dissected from *D. kuriphilus* galls from four locations of the eastern United States from May through Aug. 2006, and Jan. 2007

| Parameter                         | Site<br>$F_{3, 728}$ (P) | Time<br>$F_{4, 728}$ (P) | Site $\times$ time<br>$F_{12, 728}$ (P) |
|-----------------------------------|--------------------------|--------------------------|---|
| <i>D. kuriphilus</i> larva        | 683.2 (<0.01)            | 100.6 (<0.01)            | 67.9 (<0.01)                            |
| <i>D. kuriphilus</i> pupa         | 82.2 (<0.01)             | 31.5 (<0.01)             | 33.3 (<0.01)                            |
| <i>D. kuriphilus</i> adult        | 16.1 (<0.01)             | 31.3 (<0.01)             | 17.5 (<0.01)                            |
| Ectoparasitoid larva <sup>a</sup> | 21.2 (<0.01)             | 12.8 (<0.01)             | 20.6 (<0.01)                            |
| Parasitoid larva                  | 37.9 (<0.01)             | 162.9 (<0.01)            | 18.9 (<0.01)                            |
| Parasitoid pupa                   | 74.2 (<0.01)             | 20.9 (<0.01)             | 26.2 (<0.01)                            |
| Parasitoid adult                  | 2.9 (0.02)               | 9.5 (<0.01)              | 1.4 (0.16)                              |
| Emergence holes                   | 103.5 (<0.01)            | 55.9 (<0.01)             | 12.4 (<0.01)                            |
| Empty chambers                    | 2.26 (0.03)              | 8.3 (<0.01)              | 2.8 (<0.01)                             |
| Chamber fungi                     | 4.73 (0.01)              | 7.56 (<0.01)             | 1.87 (0.04)                             |

<sup>a</sup> Parasitoid larva and *D. kuriphilus* larva simultaneously occupying a gall chamber.

ble 2). *D. kuriphilus* were present in galls as larvae in May and June (Fig. 2A), as pupae in June (Fig. 2B), and as adults primarily in June and July (Fig. 2C). Most

adult gall wasps had emerged from galls by August, but many adults failed to emerge from galls and were still present in January (Fig. 2C). The proportion of gall chambers still containing *D. kuriphilus* adults in January was higher in galls collected at Bowling Green (14%) than in galls from other locations (0–8%) (Fig. 2C). Chambers simultaneously containing *D. kuriphilus* and parasitoid larvae were only found in Broadview Heights in May, and were absent throughout the remaining collection dates (data not shown). The proportion of chambers containing parasitoid larvae was greatest at Meadowview and Broadview Heights, and was lowest at Hiram and Bowling Green (Fig. 2D). Most parasitoid pupae were collected in January from Meadowview and Broadview Heights, but were also collected from Bowling Green in June (Fig. 2E). Parasitoid adults were not frequently observed, and were primarily present in galls at Bowling Green in May and June (Fig. 2F). The proportion of chambers with

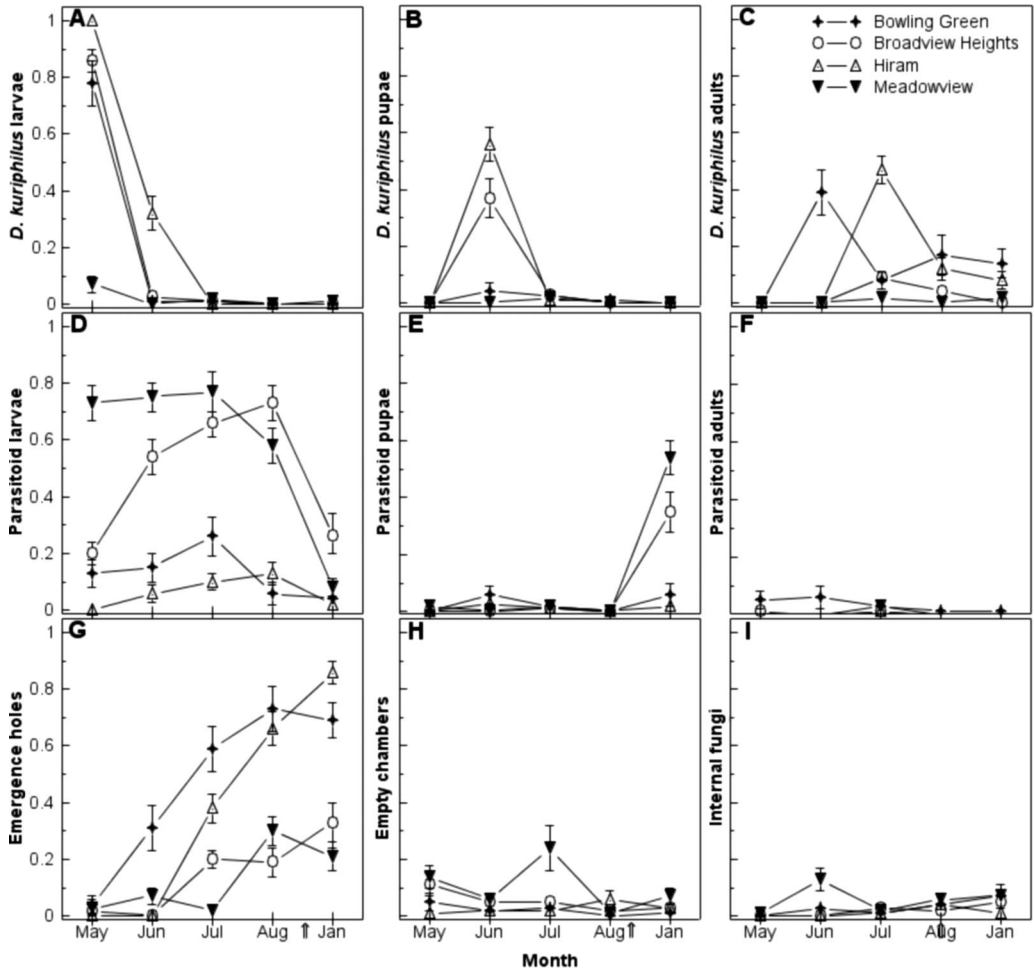


Fig. 2. Abundance and succession of inhabitants of chestnut galls collected from Bowling Green, KY (◆); Broadview Heights, OH (○); Hiram, OH (Δ); and Meadowview, VA (▼). Data represent the proportion of chambers per gall containing (A) *D. kuriphilus* larvae, (B) *D. kuriphilus* pupae, (C) *D. kuriphilus* adults, (D) parasitoid larvae, (E) parasitoid pupae, (F) parasitoid adults, (G) emergence holes, (H) empty chambers without emergence holes, and (I) internal fungal mycelia.

Table 3. Analyses of mites inhabiting the parenchyma tissues and damage caused by external gall associates of *D. kuriphilus* galls from four locations throughout the eastern United States from May through Aug. 2006, and Jan. 2007

| Parameter                   | Site                                | Time                                | Site $\times$ time                    |
|-----------------------------|-------------------------------------|-------------------------------------|---------------------------------------|
| Mites <sup>a</sup>          | $F_{3, 728} = 4.53$ ; $P = 0.01$    | $F_{3, 728} = 3.12$ ; $P = 0.03$    | $F_{3, 728} = 3.24$ ; $P = 0.01$      |
| Fungal lesions <sup>b</sup> | $\chi^2_{3, 4} = 18.9$ ; $P = 0.01$ | $\chi^2_{3, 4} = 34.4$ ; $P < 0.01$ | $\chi^2_{12, 12} = 61.5$ ; $P < 0.01$ |
| Grazing damage <sup>b</sup> | $\chi^2_{3, 4} = 402$ ; $P = 0.41$  | $\chi^2_{3, 4} = 2.7$ ; $P = 0.61$  | $\chi^2_{12, 12} = 21.2$ ; $P = 0.04$ |
| Weevil damage <sup>c</sup>  | $F_{3, 728} = 3.21$ ; $P = 0.01$    | $F_{4, 728} = 8.57$ ; $P < 0.01$    | $F_{12, 728} = 1.76$ ; $P = 0.05$     |

<sup>a</sup> No. per gall.

<sup>b</sup> Presence or absence.

<sup>c</sup> Scars per gall.

emergence holes increased throughout the collection period, but did so at the highest rate in Bowling Green and Hiram, where parasitism rates were low compared with other collection sites (Fig. 2G). Empty chambers were present throughout the year at each location, but were most abundant in galls collected from Meadowview and Broadview Heights in May, and Meadowview in July (Fig. 2H). The proportion of chambers filled with fungi was most common in galls collected from Meadowview in June and in galls collected from Meadowview, Bowling Green, and Broadview Heights in January (Fig. 2I).

Analysis of the number of mites per gall, the incidences of lesion formation and insect herbivore grazing, and the number of weevil scars per gall all revealed significant date  $\times$  site interactions (Table 3). Acarid mites inhabited the chambers and dead parenchyma tissues of galls collected at Meadowview in August, and at Bowling Green in January (Fig. 3A), but were absent during all collection dates at the

remaining collection sites. Gall lesions occurred most frequently on gall collections from Broadview Heights in July and in galls from Meadowview in June (Fig. 3B). However, the incidence of gall lesions was high at all sites in August. The occurrence of damage from insect herbivore grazing was greatest on galls collected from Meadowview in May (Fig. 3C), when it was very low at other sites. Weevil herbivory was higher on galls from Meadowview than on galls from other sites between May and August, except on galls collected from Broadview Heights, which also exhibited high levels of weevil herbivory in August (Fig. 3D).

**Canonical Correlations.** The canonical correlation analysis of internal associates and gall characteristics yielded only one significant correlation within each month (Table 4A), except January, which yielded none. In May and June, the canonical variable for internal associates (Table 4B) was correlated with *D. kuriphilus*, and the canonical variable for gall charac-

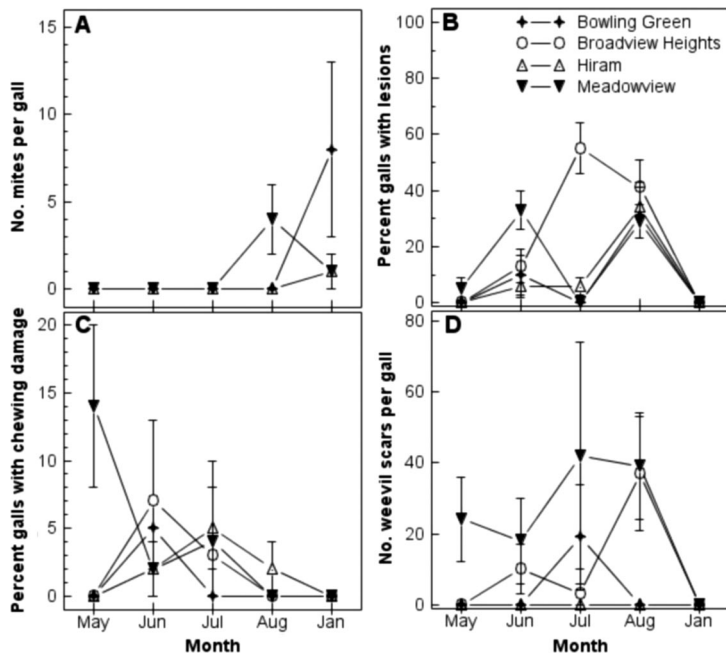


Fig. 3. Abundance and succession of mites and external gall associates collected from Bowling Green, KY (◆); Broadview Heights, OH (○); Hiram, OH (Δ); and Meadowview, VA (▼). Data represent (A) the number of mites per gall, (B) percentage of galls per tree containing external fungal lesions or (C) insect grazing damage, and the (D) number of weevil-feeding sites per gall.

Table 4. Significant canonical correlations between internal gall associates and gall characteristics

| Canonical variables  | Canonical correlation         |                               |                               |                               |
|--|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
|  | May                           | June                          | July                          | Aug.                          |
| A. Internal associates vs gall characteristics                                     |                               |                               |                               |                               |
| Correlation  | 0.38                          | 0.38                          | 0.46                          | 0.47                          |
| Significance   | $F_{10, 294} = 2.6; P < 0.01$ | $F_{10, 248} = 2.4; P < 0.01$ | $F_{10, 278} = 4.5; P < 0.01$ | $F_{10, 260} = 3.7; P < 0.01$ |
| B. Correlations between the following internal associates and gall characteristics |                               |                               |                               |                               |
| <i>D. kuriphilus</i>   | 0.43                          | 0.70                          | -0.11                         | -0.16                         |
| Parasitoids  | -0.01                         | -0.18                         | 0.72                          | 0.98                          |
| Emergence  | -0.07                         | -0.61                         | -0.93                         | -0.78                         |
| Empty  | -0.01                         | -0.08                         | 0.07                          | -0.17                         |
| Chamber fungi  | -0.01                         | -0.65                         | -0.11                         | -0.24                         |
| C. Correlations between the following gall characteristics and internal associates |                               |                               |                               |                               |
| Volume   | 0.80                          | 0.57                          | 0.17                          | 0.20                          |
| Schlerenchyma  | 0.87                          | 0.94                          | 0.99                          | 0.96                          |

teristics (Table 4C) was correlated with both gall volume and schlerenchyma thickness. Thus, these canonical correlations demonstrate a relationship among *D. kuriphilus*, gall volume, and a thick schlerenchyma layer in May and June. In addition to these relationships, in June the canonical variable for internal associates was negatively correlated with chamber fungi and emergence, and showed a weak negative correlation with parasitism, thus demonstrating a negative relationship between these variables and gall volume and thick schlerenchyma. In July and August, the canonical variable for internal associates was positively correlated with parasitoids, negatively correlated with emergence, and showed a weak, but negative correlation with *D. kuriphilus* and chamber fungi (Table 4B). The canonical variable for gall characteristics was highly correlated with schlerenchyma thickness and weakly correlated with gall size (Table 4C). Thus, these canonical correlations demonstrate a relationship between parasitoids and thick schlerenchyma layers in July and August.

The canonical correlation analysis of external associates and gall characteristics yielded only one significant correlation identified in July and August (Table 5A). In July, the canonical variable for external associates was highly correlated with fungal lesions (Table

5B), and the canonical variable for gall characteristics was highly correlated with gall volume and weakly correlated with stem galls (Table 5C). This canonical correlation demonstrates a positive relationship between fungal lesions and gall size in July. In August, the canonical variable for external associates was correlated with fungal lesions, weakly correlated with weevil damage, and weakly, but negatively correlated with herbivore grazing damage (Table 5B). The canonical variable for gall characteristics was associated with both the incidence of stem galls and gall volume (Table 5C). Thus, these canonical correlations demonstrate a positive relationship among fungal lesions, stem galls, and gall size, and a negative relationship among grazing damage, stem galls, and gall size in August.

The canonical correlation analysis of internal and external gall associates yielded one significant relationship in May, June, and July (Table 6A). In May, the canonical variable for internal associates was positively correlated with parasitoids and chamber fungi, and negatively correlated with *D. kuriphilus* (Table 6B). The canonical variable for external associates was correlated with all three external associates (Table 6C). In June, the canonical variable for internal associates was highly correlated with *D. kuriphilus*, and

Table 5. Significant canonical correlations between external gall associates and gall characteristics

| Canonical variables  | Canonical correlation         |                              |
|--|-------------------------------|------------------------------|
|  | July                          | Aug.                         |
| A. External associates vs gall characteristics                                     |                               |                              |
| Correlation  | 0.70                          | 0.34                         |
| Significance   | $F_{6, 282} = 18.9; P < 0.01$ | $F_{6, 264} = 3.3; P < 0.01$ |
| B. Correlations between the following external associates and gall characteristics |                               |                              |
| Lesions  | 0.99                          | 0.85                         |
| Weevil damage  | 0.01                          | 0.44                         |
| Grazing damage   | 0.07                          | -0.46                        |
| C. Correlations between the following gall characteristics and external associates |                               |                              |
| Volume   | 0.99                          | 0.70                         |
| Stem galls   | 0.26                          | 0.83                         |

Table 6. Significant canonical correlations between internal gall associates and external gall associates

| Canonical variables   | Canonical correlation         |                               |                               |
|---|-------------------------------|-------------------------------|-------------------------------|
|   | May                           | June                          | July                          |
| A. Internal associates vs external associates                                     |                               |                               |                               |
| Correlation   | 0.44                          | 0.33                          | 0.44                          |
| Significance  | $F_{15, 409} = 1.5; P < 0.01$ | $F_{15, 389} = 1.5; P < 0.01$ | $F_{15, 381} = 2.5; P < 0.01$ |
| B. Correlations between the following internal associates and external associates |                               |                               |                               |
| <i>D. kuriphilus</i>  | -0.72                         | 0.91                          | -0.36                         |
| Parasitoids   | 0.72                          | -0.84                         | 0.29                          |
| Emergence   | -0.09                         | -0.07                         | -0.06                         |
| Empty   | -0.09                         | -0.10                         | -0.10                         |
| Chamber fungi   | 0.60                          | -0.01                         | 0.63                          |
| C. Correlations between the following external associates and internal associates |                               |                               |                               |
| Lesions   | 0.45                          | -0.98                         | 0.96                          |
| Weevil damage   | 0.76                          | -0.19                         | 0.11                          |
| Grazing damage  | 0.63                          | 0.06                          | -0.08                         |

negatively correlated with parasitoids (Table 6B). The canonical variable for external associates showed a strong negative correlation with fungal lesions and a weak negative correlation with weevil damage (Table 6C). In July, the canonical variable for internal associates was correlated with internal fungi and weakly correlated with parasitoids, and showed a weak negative correlation with *D. kuriphilus* (Table 6B). The canonical variable for external associates was highly correlated with fungal lesions and weakly correlated with weevil damage (Table 6). Thus, these canonical correlations demonstrate a negative relationship between the presence of *D. kuriphilus* and grazing damage in May, a strong negative relationship between the presence of *D. kuriphilus* and fungal lesions from May through July, and a weak negative relationship between the presence of *D. kuriphilus* and damage caused by weevil herbivory from May through July.

Discussion

Parasitoids were present in up to 80% of gall chambers, and were the most frequent cause of mortality for *D. kuriphilus* in our study. The opposing signs for *D. kuriphilus* and parasitoids revealed in each canonical correlation analysis (Tables 4–6) emphasize the negative effects of parasitism on *D. kuriphilus*. The lowest rates of parasitism were recorded at the Bowling Green and Hiram locations. In Bowling Green, parasitoids were generally observed as adults within galls collected in May and June, which was sooner than observed at other locations. A previous study determined that the primary parasitoid at Bowling Green was the native parasitoid, *O. labotus*, whereas the primary parasitoid at Broadview Heights and Meadowview was the introduced parasitoid, *T. sinensis* (Cooper and Rieske 2007). *T. sinensis* has a long evolutionary history with *D. kuriphilus* compared with *O. labotus*, and therefore, may be better adapted to parasitize *D. kuriphilus* galls. Conversely, the association between *O. labotus* and *D. kuriphilus* is more recent. Thus, parasitism by *O. labotus* may be limited in *D. kuriphilus* galls, for example, to certain gall size classes. At Hiram, the *D. kuriphilus* population is a

relatively recent occurrence, and robust parasitoid populations may not yet be established at this location. Parasitism was influenced by variations in gall characteristics. Overall, parasitism was negatively correlated with gall size and schlerenchyma thickness before *D. kuriphilus* emergence in June/July. Increased gall size caused by multilocularity (many larval chambers in a single gall) is thought to represent a defensive strategy against parasitism by increasing the gall wasps' influence over the tree's physiology and gall induction (Stone and Schonrogge 2003). The schlerenchyma layer is the toughest gall tissue (Cooper and Rieske 2009) and may provide the gall wasp with protection against parasitoid penetration. However, parasitoids were positively correlated with gall size and schlerenchyma thickness in July and August (after *D. kuriphilus* emergence). This suggests that parasitoids may select chambers with thicker schlerenchyma, but ovipositor length may limit their access to galls with schlerenchyma of intermediate thickness, or to a specific time frame in schlerenchyma layer development when galls are most accessible (Stone et al. 2002). Empty chambers, which constituted a fairly consistent 25% of the total throughout our sampling, apparently represent early season death of the chamber inhabitants (either *D. kuriphilus* or parasitoid). Although the cause of death resulting in empty chambers is unknown, parasitoids are thought to be involved (Kato and Hijii 1999). Kato and Hijii (1999) proposed that empty chambers were caused from adult feeding by an early strain of *Torymus beneficus* Yasumatsu, but this parasitoid has not been identified in North America, and empty chambers were common in Bowling Green, where *Torymus* spp. were reportedly not abundant (Cooper and Rieske 2007; unpublished data). Alternatively, either unidentified mechanisms of host plant resistance or overcrowding when gall wasps and parasitoids occur simultaneously within individual chambers may cause mortality associated with empty chambers (Cooper and Rieske 2009). Finally, the formation of empty chambers may be a defensive decoy strategy against parasitoids (Stone and Schonrogge 2003). In fact, canonical correlation analysis revealed



a negative correlation between parasitoids and empty chambers in August, but this relationship was not detected during any other collection date. The cause and significance of empty gall chambers deserve more attention in future studies.

Many chambers (up to 14%) were filled with white fungal mycelia. The chamber fungi are unidentified, and it is unknown whether they are pathogens of the gall wasp or saprophytes of wasp cadavers (Williams 1994). Chamber fungi first appeared in galls in June and gradually increased in abundance over time. Canonical correlations revealed that the presence of chamber fungi was negatively correlated with gall volume and schlerenchyma thickness. There was also a negative correlation between *D. kuriphilus* and chamber fungi, but no relationship between fungi and parasitoids, indicating that chamber fungi occur in chambers containing the gall inducer and not the parasitoids. Paramount to understanding the interactions between chamber fungi and the gall inducer is the identification of the fungus and its ecological role (pathogenic or saprophytic).

Another source of *D. kuriphilus* mortality is the failure of adult wasps to emerge from gall tissues (Otake 1980, 1989). Gall wasp adults failed to emerge from up to 14% of chambers in our study. Many of these adult gall wasps chewed partial tunnels exiting the gall chamber, but were observed dead in August and January (Fig. 1C). Significantly more gall wasps failed to emerge from galls collected in Bowling Green (American chestnut) and Broadview Heights (Chinese chestnut), than from the remaining sites (hybrids), but the reasons are unknown.

*D. kuriphilus* presence was inversely correlated with the presence of fungal lesions on the gall exteriors, whose identity and relatedness to the internal chamber fungi are unknown. Indirect mortality for many oak galling cynipids is associated with the formation of similar black lesions produced by endophyte fungi on the exterior of galls (Taper and Case 1987, Carroll 1995, Wilson 1995). Although lesions and *D. kuriphilus* were negatively correlated, we could not determine whether lesions caused gall wasp mortality, or whether changes in gall chemistry (Mani 1964) after gall wasp mortality propagated lesion development. Parasitoids were positively correlated with fungal lesions. Ovipositing adult parasitoids may have acted as fungal vectors, created an infection court for opportunistic fungi, or caused damage that induced pathogenicity from endophytes. Identification of the causal fungus could potentially lead to the development of biological controls in cultivated chestnut.

*D. kuriphilus* galls are also exploited by the lesser chestnut weevil in North America (Cooper and Rieske 2007). This weevil causes substantial feeding damage to nuts, and is among the most serious pests of cultivated chestnut (Wahl 2002). This weevil emerges from overwintering sites in the spring, but its diet before nut development is not well understood (Keesey and Barrett 2008). Weevil feeding was positively correlated with stem galls and large gall size in our study. Large stem galls may have been visually per-

ceived as developing chestnuts by foraging weevils. Weevil feeding was first recorded in Meadowview in May and in Broadview Heights in June, but increased in August when nuts were present. It is unknown whether weevils benefit from feeding on galls. If the galls provide weevils with an early season nutritive source, high gall wasp infestations could lead to greater weevil survival and increased outbreaks. This potential relationship warrants further investigation.

Cynipid galls are commonly damaged by incidental insect grazing (Schultz 1992). Up to 15% of *D. kuriphilus* galls were damaged by insect herbivore grazing in our study, but the insect herbivores that caused this damage have not been identified. Leaves attached directly to galls are more suitable for gypsy moth caterpillar (*Lymantria dispar* L., Lepidoptera: Lymantriidae) growth compared with foliage without galls, and this increased suitability may increase the incidence of herbivore grazing (Cooper and Rieske 2009). Damage caused by herbivore grazing was negatively correlated with stem galls in August, which suggests that leaf galls, which are surrounded entirely by foliage, are at greater risk from grazing herbivores. Both weevil damage and herbivore grazing damage were negatively correlated with *D. kuriphilus* and positively correlated with parasitoids and empty chambers. These correlations suggest that changes in gall chemistry in the absence of *D. kuriphilus* (empty chambers or chambers containing parasitoids) increase the risk of herbivory on gall exteriors, but we cannot discount herbivory as a cause of *D. kuriphilus* mortality.

Acarid mites appeared in galls collected from Meadowview in August and in Bowling Green in January. They inhabited the empty chambers and parenchyma layers of withered desiccated galls, suggesting they are saprophytic on dead gall tissues or use dead galls as an overwintering site. The extent to which these mites interact with other gall associates, including overwintering parasitoids, is unknown.

In conclusion, our study provides important insight into the ecological interactions among chestnut, an invasive cynipid gall wasp, and parasitoids and external associates of the gall wasp. Our study provides evidence that natural variation in gall traits affects mortality factors of *D. kuriphilus*, including parasitism. Future studies should investigate to what extent variations among gall characteristics are caused by plant or wasp phenotypes. This is the first study to quantitatively relate schlerenchyma layer thickness to cynipid mortality factors and to indicate that the schlerenchyma layer contributes to protecting the gall wasp. These observations provide direct support for the enemy hypothesis, which postulates that galls provide gall wasps with protection from natural enemies. This study improves our understanding of *D. kuriphilus* ecology, which is critical to the future development of management strategies against this pest.

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## References Cited

- Aebi, C. Y., K. Schonrogge, C. Melika, A. Alma, G. Bosio, A. Quacchia, L. Picciau, Y. Abe, S. Moriya, K. Yara, G. Seljak, and G. M. Stone. 2006. Parasitoid recruitment to the globally invasive chestnut gall wasp, pp. 103–121. In K. Ozaki, J. Yakawal, T. Ohgushi, and P. W. Price (eds.), Ecology and evolution of galling arthropods and their associates. Springer, Tokyo, Japan.
- Allison, S. D., and J. C. Schultz. 2005. Biochemical responses of chestnut oak to a galling cynipid. *J. Chem. Ecol.* 31: 151–166.
- Anagnostakis, S. L. 2001. The effect of multiple importations of pests and pathogens on a native tree. *Biol. Invasions* 3: 245–254.
- Anagnostakis, S. L., S. Clark, and H. McNab. 2009. Preliminary report on segregation of resistance in chestnuts to infestation by Oriental chestnut gall wasp. *Acta Hort.* 815: 33–35.
- Bailey, R., K. Schönrogge, J. M. Cook, G. Melika, G. Csóka, C. Thuróczy, and G. N. Stone. 2009. Host niches and defensive extended phenotypes structure parasitoid communities. *PLoS Biol.* 7: e1000179. (doi:10.1371/journal.pbio.1000179).
- Carroll, G. 1995. Forest endophytes: pattern and process. *Can. J. Bot.* 73: S1316–S1324.
- Cooper, W. R., and L. K. Rieske. 2007. Community associates of an exotic gallmaker, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), in Eastern North America. *Ann. Entomol. Soc. Am.* 100: 236–244.
- Cooper, W. R., and L. K. Rieske. 2009. Woody stem galls interact with foliage to affect community associations. *Environ. Entomol.* 38: 417–424.
- Cornell, H. V. 1983. The secondary chemistry and complex morphology of galls formed by the Cynipinae (Hymenoptera): why and how. *Am. Midl. Nat.* 110: 225–234.
- Eliason, A. E., and D. A. Potter. 2000. Budburst phenology, plant vigor, and host genotype effects on the leaf-galling generation of *Callirhytis cornigera* (Hymenoptera: Cynipidae) on pin oak. *Popul. Ecol.* 29: 1199–1207.
- Felt, E. P. 1940. Plants galls and gall makers. Comstock, New York.
- Hartley, S. E. 1998. The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall-former? *Oecologia* 113: 492–501.
- Hartley, S. E., and J. H. Lawton. 1992. Host plant manipulation by gall insects: a test of the nutrition hypothesis. *J. Anim. Ecol.* 61: 113–119.
- Hebard, F. V. 1994. Inheritance of juvenile leaf and stem morphological traits in crosses of Chinese and American chestnut. *J. Hered.* 85: 440–446.
- Ito, M., and N. Hijii. 2002. Factors affecting refuge from parasitoid attack in a cynipid wasp, *Aphelonox glanduliferae*. *Popul. Ecol.* 44: 23–32.
- Ito, M., and K. Ozaki. 2005. Response of a gall wasp community to genetic variation in the host plant *Quercus crispula*: a test using half-sib families. *Acta Oecologica* 27: 17–24.
- Kato, K., and N. Hijii. 1997. Effects of gall formation by *Dryocosmus kuriphilus* Yasumatsu (Hym., Cynipidae) on the growth of chestnut trees. *Jpn. J. Appl. Entomol.* 121: 9–15.
- Kato, K., and N. Hijii. 1999. Mortality factors of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) after gall formation. *Entomol. Sci.* 2: 483–491.
- Keesey, I. W., and B. A. Barrett. 2008. Seasonal occurrence and soil distribution of the lesser chestnut weevil, *Curculio sayi* (Coleoptera: Curculionidae) in Mid Missouri. *J. Kansas Entomol. Soc.* 81: 345–354.
- Kinsey, A. C. 1920. Phylogeny of Cynipid genera and biological characteristics. *Am. Mus. Nat. Hist. Bull.* 42: 357–402.
- Kinsey, A. C. 1935. The economic importance of the Cynipidae. *J. Econ. Entomol.* 28: 86–91.
- Mani, M. S. 1964. Ecology of plant galls. Dr. W. Junk Publisher, The Hague.
- Otake, A. 1980. Chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae): a preliminary study on trend of adult emergence and some other ecological aspects related to the final stage of its life-cycle. *Appl. Entomol. Zool.* 15: 96–105.
- Otake, A. 1989. Chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae): analyses of records on cell contents inside galls and on emergence of wasps and parasitoids outside galls. *Appl. Entomol. Zool.* 24: 193–201.
- Paillet, F. L. 1982. The ecological significance of American chestnut (*Castanea dentata* [Marsh] Borkh) in the Holocene forests of Connecticut. *Bull. Torrey Bot. Club* 109: 457–473.
- Paillet, F. L. 2002. Chestnut: history and ecology of a transformed species. *J. Biogeogr.* 29: 1517–1530.
- Payne, J. A., A. S. Menke, and P. M. Schroeder. 1975. *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), an Oriental chestnut gall wasp in North America. *USDA Coop. Econ. Insect Rep.* 25: 903–905.
- Price, P. W., G. W. Fernandes, and G. L. Waring. 1987. Adaptive nature of insect galls. *Environ. Entomol.* 16: 15–24.
- Scheiner, S. M. 2001. MANOVA: multiple response variables and multispecies interactions, pp. 104–105. In S. M. Scheiner and J. Gurevitch (eds.), Design and analysis of ecological experiments. Oxford University Press, Oxford, New York.
- Schultz, B. B. 1992. Insect herbivores as potential causes of mortality and adaptation in gall forming insects. *Oecologia* 90: 297–299.
- Shorthouse, J. D. 1973. The insect community associated with rose galls of *Diplolepis polita* (Cynipidae: Hymenoptera). *Quaestiones Entomol.* 9: 55–98.
- Shorthouse, J. D. 1993. Adaptations of galls wasp of the genus *Diplolepis* (Hymenoptera: Cynipidae) and the role of gall anatomy in cynipid systematics. *Mem. Entomol. Soc. Can.* 165: 139–163.
- Stehli, B. 2006. Oriental gall wasp in Ohio: an update. *The Nutshell* 60: 27.
- Stone, G. N., and K. Schonrogge. 2003. The adaptive significance of insect gall morphology. *Trends Ecol. Evol.* 18: 512–522.
- Stone, G. N., K. Schonrogge, R. J. Atkinson, D. Bellido, and J. Pujade-Villar. 2002. The population biology of oak gall

- wasps (Hymenoptera: Cynipidae). *Annu. Rev. Entomol.* 47: 633–664.
- Sumerford, D. V., W. G. Abrahamson, and A. E. Weis. 2000.** The effects of drought on the *Solidago altissima*-*Eurosta solidaginis*-natural enemy complex: population dynamics, local extirpations, and measures of selection intensity on gall size. *Oecologia* 122: 240–248.
- Taper, M. L., and T. J. Case. 1987.** Interactions between oak tannins and parasite community structure: unexpected benefits of tannins to cynipid gall-wasps. *Oecologia* 71: 254–261.
- Wahl, T. 2002.** The chestnut grower's primer. Southeast Iowa Nut Growers, Wapello, IA.
- Washburn, J. O., and H. V. Cornell. 1981.** Parasitoids, patches, and phenology: their possible role in the local extinction of a cynipid gall wasp population. *Ecology* 62: 1597–1607.
- Williams, M.A.J. 1994.** Plant galls: a perspective, pp. 1–6. *In* M.A.J. Williams (ed.), *Plant galls: organisms, interactions, populations*. Oxford University Press, New York, NY.
- Wilson, D. 1995.** Fungal endophytes which invade insect galls: insect pathogens, benign saprophytes, or fungalinquilines. *Oecologia* 103: 255–260.

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